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Additional records of *Volvariella dunensis* (*Basidiomycota*, *Agaricales*): morphological and molecular characterization

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Abstract — Collections morphologically assignable to *Volvariella dunensis* from Sardinia and the Atlantic coast of Spain were revised and compared with the original collections. Molecular data supporting all examined collections as *V. dunensis* expand its known geographic distribution. A revised morphological characterization and a phylogenetic analysis of all *Volvariella* species sequenced to date are provided.

Key words — *Agaricomycetes,* ITS, phylogeny, *Volvariella volvacea*

Introduction

The genus *Volvariella* Speg., which is composed of saprotrophic or mycotrophic agarics, has been historically considered a member of the family *Pluteaceae* Kotl. & Pouzar (Singer 1986). The genus is characterized macroscopically by a pink spore-print, free lamellae, and a universal veil that forms a saccate volva at the base of the stipe and microscopically by the inverse hymenophoral trama (Singer 1986). Results from earlier molecular studies (Moncalvo et al. 2002, Matheny et al. 2006) led to questions about its monophyly and phylogenetic position in the *Pluteaceae*. More recent research focused on the *Pluteaceae* (Justo et al. 2011) showed that *Volvariella* is polyphyletic with two major clades. The large-spored, viscid species belonging to the *V. gloiocephala* complex were resolved as the sister group of *Pluteus* Fr. and accommodated under the new generic name *Volvopluteus* Vizzini et al. *Volvariella*, the remaining clade, is therefore restricted to the dry, small-spored species. *Volvariella* falls outside the pluteoid clade and based on data from ribosomal loci (nSSU, 5.8S, nLSU) shows affinities to some hygrophoroid

genera (*Cuphophyllus* (Donk) Bon, *Cantharocybe* H.E. Bigelow & A.H. Sm.), but its phylogenetic position within the *Agaricales* is still unclear.

Volvariella dunensis has been the subject of a detailed taxonomic study based on the re-examination of the original collections from the Mediterranean coast of Spain (Justo & Castro 2010). Very recently, a collection assignable to *V. dunensis* was reported from the Atlantic coast of Spain (Fernández-Sasia 2010 as "*Volvariella nigrovolvacea* var. *dunensis*"). Also, the identity of the Sardinian collection reported by Contu & La Rocca (1999) as *Volvariella nigrovolvacea* Kosina was recently questioned (Justo & Castro 2010), and it comes close to the morphological concept of *V. dunensis*.

The purpose of this study was to determine whether the collections reported by Fernández-Sasia (2010) and Contu & La Rocca (1999) represent *V. dunensis* using both morphological and molecular characters (ITS region).

Materials & methods

Morphology

Descriptive terminology follows Vellinga (1988) and Boekhout (1990). Microscopical preparations from dried material were mounted in Congo Red and 5% KOH. The following abbreviations are used in text: avl for average length, avw for average width, Q for quotient of length and width and avQ for average quotient. Extreme measurements are indicated within parentheses. Herbarium acronyms follow Thiers (2011) except that "SCM" is used for the "Societat Catalana de Micologia" herbarium and RFS refers to the personal herbarium of Roberto Fernández-Sasia.

Molecular analysis

Standard methods for DNA extraction, PCR amplification and DNA sequencing were applied (e.g. Justo et al. 2011). Primer pairs ITS1F and ITS4 (Gardes & Bruns 1993) were used for both PCR and sequencing. Complete information about the collections of *Volvariella* that were sequenced is given in Tab. 1. The remaining sequences used in the analysis were retrieved from GenBank and come from the studies of Menolli & Capelari (2008), Li et al. (2009) and Justo et al. (2011). GenBank accession numbers are given in Fig. 2. No suitable outgroup for *Volvariella* was found using BLAST searches (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Therefore, after running a preliminary analysis using *Coprinus comatus* (O.F. Müll.) Pers. as an outgroup for all *Volvariella* sequences and taking into consideration the results from the combined nLSU-5.8S-nSSU analyses of Justo et al. (2011), *V. bombycina* (Schaeff.) Singer and *V. volvacea* (Bull.) Singer were used as outgroup taxa in the final dataset. These two species appear to be the sister-group to all other species of *Volvariella* sequenced to date. Sequences were aligned with MAFFT (Katoh et al. 2002) using the Q-INS-i strategy. The alignment was examined and manually corrected in MacClade 4.05 (Maddison & Maddison 2002). It has been deposited in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S11441). Pairwise similarities were calculated using Jalview (Waterhouse et al. 2009). A Maximum Likelihood analysis was run in RAxML servers (Stamatakis et al. 2008) with 100 rapid bootstrap replicates.

Table 1. *Volvariella* samples newly sequenced in this study.

Species	Country—location	Collector; sampling date [herb. number]	Habitat
V. dunensis (Vila et al. 1999, Justo & Castro 2010)	Spain—Barcelona, El Prat de Llobregat, El Pinar	F. Àngel; 08.II.1997 [SCM 3513]	Open dunes
V. dunensis (Fernández-Sasia 2010)	Spain—Bizkaia, Muskiz	R. Fernández-Sasia; 10.III.2007 [RFS 070310-03]	Dunes with *Tamarix gallica & Carpobrotus edulis*
V. dunensis (Contu & La Rocca 1999 as *V. nigrovolvacea*)	Italy—Sardinia, prov. Cagliari, Chia	M. Contu; 26.XII.1997 [TO-AV140]	Sand dune in *Juniperus phoenicea* debris; among *Pistacia lentiscus*, *J. phoenicea, J. oxycedrus*
V. hypopithys (Fr.) Shaffer	Italy—Stazzo Montesu, Olbia-Tempio	M. Contu; 26.XI.2002 [TO-AV137]	Under *Quercus suber*
V. pusilla (Pers.) Singer	Spain—Asturias, Endriga	R. Picón; 5.X.2005 [AJ51 (LOU)]	Among grasses
V. pusilla	Italy—Oschiri, Olbia-Tempio	M. Contu; 11.XI.2000 [TO-AV139]	Among grasses
V. strangulata (Romagn.) Vizzini & Contu	Italy—Emilia Romagna	L. Setti; — [TO-AV141]	Not recorded
V. terrea	France—Rixheim	E. Musumeci; 01.X.2005 [LUG 11010, holotypus]	Under *Carpinus betulus* near *Agaricus xanthodermus*
Volvariella sp.	Spain—Madrid, Parque del Oeste	L.A. Parra; 6.VIII.2005 [LOU 18294]	Among *Cedrus atlantica* remnants
Volvariella sp.	Slovenia—Tolminski, Tolmin	J. Kavcic; 12.VII.2007 [TO-AV143]	Under *Fagus sylvatica* and *Fraxinus* sp.

Taxonomy

ITS sequences from the collections TO-AV140 (Sardinia) and RFS 070310-03 (Atlantic coast of Spain) are 99.5% and 100% identical to the ITS of SCM 3513 (Mediterranean coast of Spain), respectively. All collections are therefore considered to represent *V. dunensis*.

Macromorphological differences between the Sardinian and Atlantic collections with respect to the original collections of *V. dunensis* are subtle, but they concern characters commonly used to delimitate species in *Volvariella* such as pileus diameter, stipe colour and volva morphology and colour. Variation in

Figure 1. *Volvariella dunensis*. Basidiomes (Sardinia, Italy, TO AV140). Bar = 50 mm.

microscopical characters is also subtle and mainly concerns size and shape of basidiospores and cystidia. Here we provide a revised description of *V. dunensis* based on all the collections known so far, highlighting only those characters that differ from the description provided in Justo & Castro (2010).

Volvariella dunensis (Vila, Àngel & Llimona) Justo & M.L. Castro, Mycotaxon 112: 262. 2010. Fig. 1

≡ *Volvariella nigrovolvacea* var. *dunensis* Vila, Àngel & Llimona, Revista Catalana de Micologia 22: 131. 1999.

Pileus diameter 15–100 mm. Stipe surface white to grey (similar to pileus colour). Volva white to externally dark sepia to brownish-black, entire or lobate. Basidiospores (6–)6.5–9.0 × 4.5–6(–6.7) µm, avl × avw = 7.5–7.9 × 5.1–5.5 µm, Q = (1.15–)1.3–1.7(–1.8), avQ = 1.4–1.55, ellipsoid to oblong, rarely broadly ellipsoid. Pleurocystidia (34–)40–95(–112) × 13.5–45(–50) µm, clavate, (narrowly) utriform, obovoid, more rarely broadly lageniform or fusiform; colourless; with thin, smooth walls, a few with a slightly thickened wall (up to 0.8 µm thick). Cheilocystidia 20–80(–120) × 15–60 µm, clavate, utriform, fusiform or lanceolate, without apical appendages, colourless; with thin, smooth walls, a few with a slightly thickened wall (up to 0.8 µm thick). Caulocystidia only observed in the original collections, absent in TO-AV140 and RFS 070310-03.

Habit, habitat and distribution — Gregarious, usually with most of the basidiomes growing deeply buried in the sand. In coastal dunes. Known from the Mediterranean basin (NE Spain, Sardinia) and the Atlantic coast of Spain.

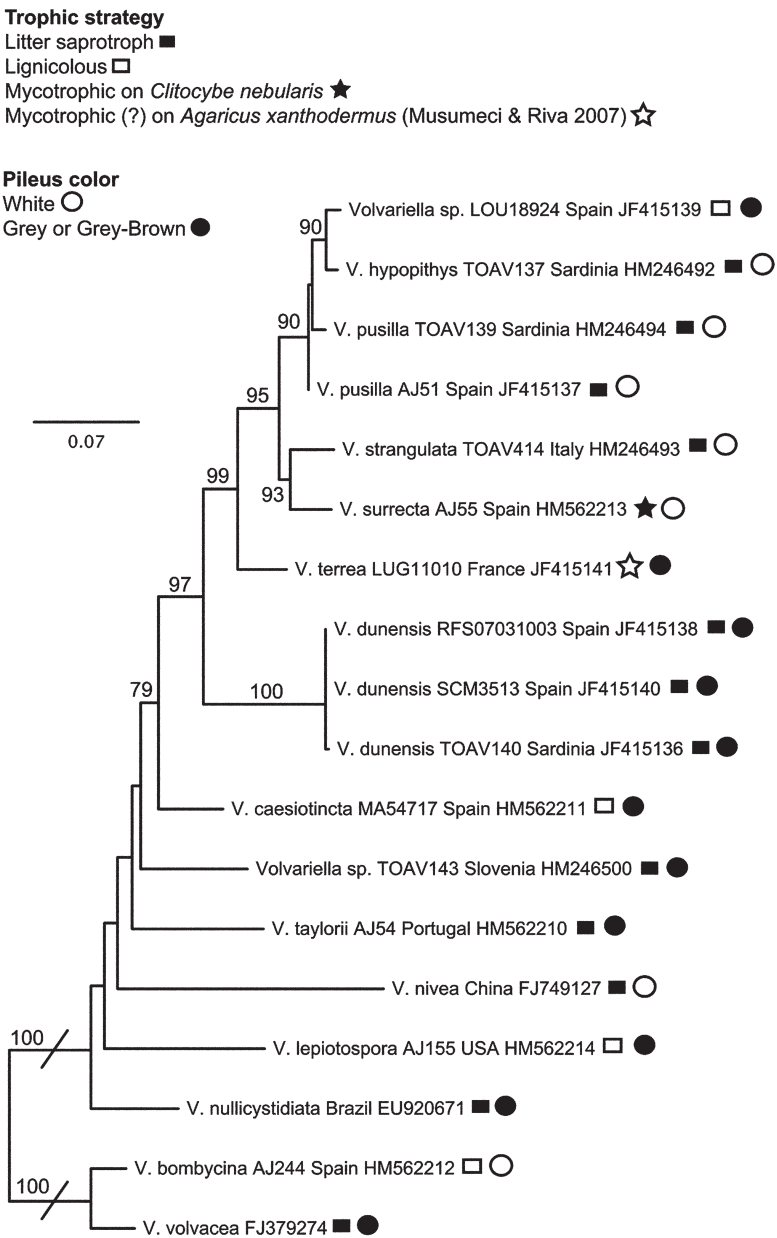

Figure 2. Best tree from the Maximum Likelihood analysis of ITS sequences of *Volvariella*. Bootstrap values above 70% are indicated on the branches. The root length has been reduced to facilitate graphical representation. Scale bar value indicates nucleotide substitutions/site.

Phenology — *Volvariella dunensis* seems to have a relatively narrow and particular time of fructification during the winter, spanning from the last days of December (Sardinia) to early March (Atlantic coast of Spain).

Comments—The arenicolous habitat, winter fructification, and cystidial size and morphology are the key characters for separating *V. dunensis* from other species with grey-fibrillose pilei, especially *V. volvacea*. Courtecuisse (1984) reports that *V. volvacea* could be collected "au niveau des dunes" in the Atlantic coast of France, and Bon & Gehu (1973) signal this species as typical of the *Ammophiletea* communities. In the UK (Orton 1986), Netherlands (Boekhout 1990), and Spain (Justo & Castro 2010), *V. volvacea* has been consistently reported as growing inside greenhouses or outside on organic rich substrates (leaves, compost, sawdust). Given the external resemblance of both species it is very likely, in our opinion, that at least some coastal records of *V. volvacea* from France may, in fact, represent *V. dunensis*.

In the phylogenetic analysis, *V. dunensis* appears as sister to the clade containing *V. terrea* Musumeci & A. Riva and the *V. pusilla* group (Fig. 2). Apart from this relationship, the internal nodes of the tree receive generally low support and many species appear on relatively long branches. This is probably a consequence of the high level of divergence in the ITS sequences of *Volvariella*. For instance, the relatively low 62% pairwise similarity between *V. bombycina* and *V. nivea* T.H. Li & Xiang L. Chen could indicate either a high rate of molecular change in the genus or a relatively ancient speciation event.

The number of *Volvariella* taxa and collections sampled for molecular analyses is still insufficient to make broad generalizations about the morphological species-rank taxonomy. However, it seems that many characters traditionally used for grouping species in the genus such as pileus colour (grey-brown vs. white) or trophic strategy (litter saprotroph, lignicolous, mycotrophic) have undergone many transitions during the evolution of *Volvariella* species (see character states on Fig. 2). Additional research using both morphological and molecular data is badly needed to gain further insights in the taxonomy and evolution of the genus.

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